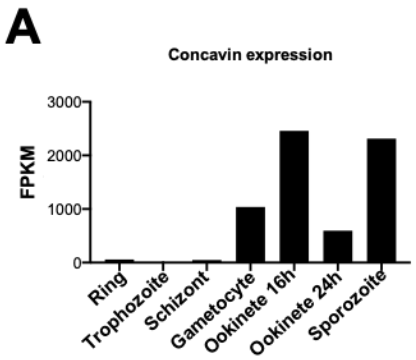
Appendix

Appendix Figure S1



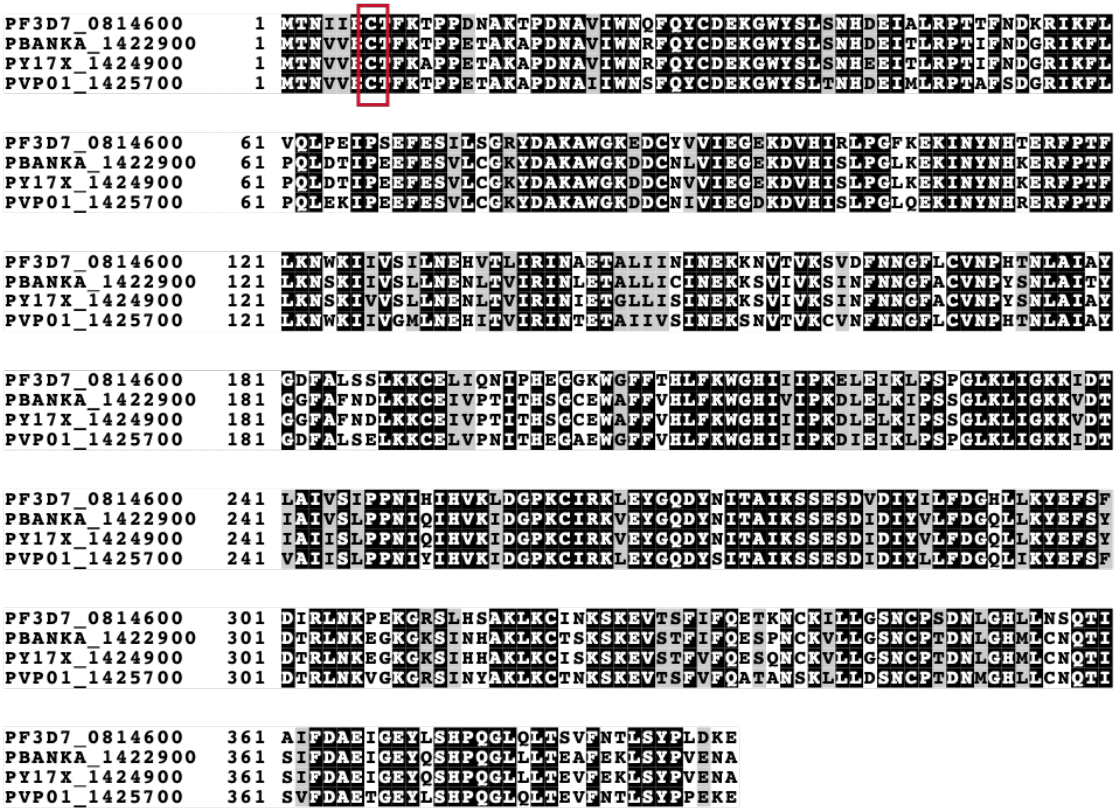
B

		Gene ID	Identity Pb (%)
P. berghei	393aa	PBANKA_1422900	100
P. yoelii	393aa	PY17X_1424900	96
P. vivax	393aa	PVP01_1425700	80
P. falciparum	393aa	PF3D7_0814600	76
T. gondii	400aa	TGGT1_216650	38

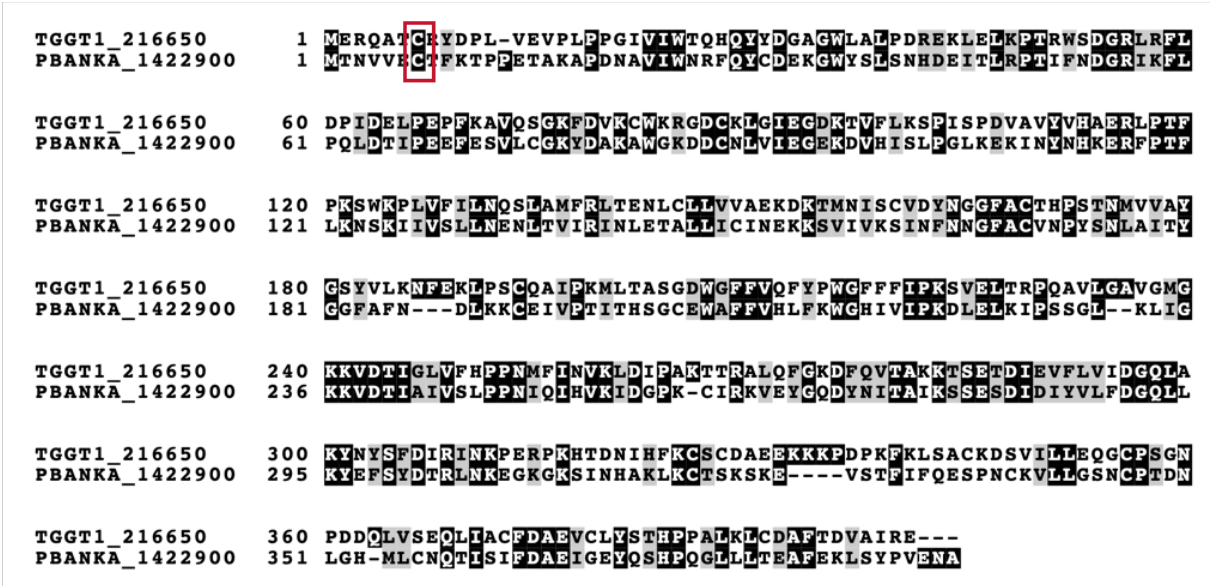
Appendix Figure S1 | (A) RNAseq abundance of concavin in blood and mosquito stage parasites **(B)** Sequence identity of *P. berghei* concavin with *P. yoelii*, *P. vivax*, *P. falciparum* and *T. gondii*.

Appendix Figure S2

A

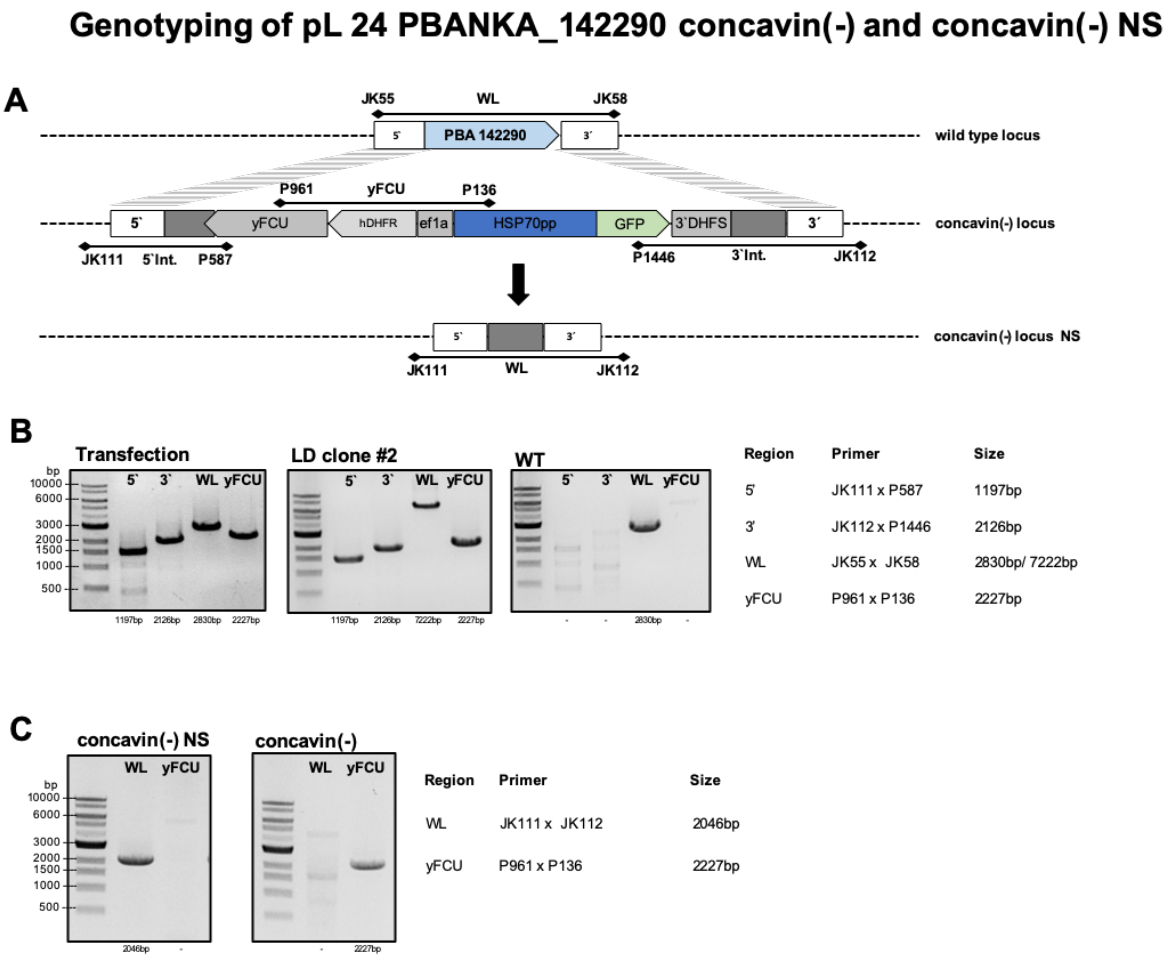


B



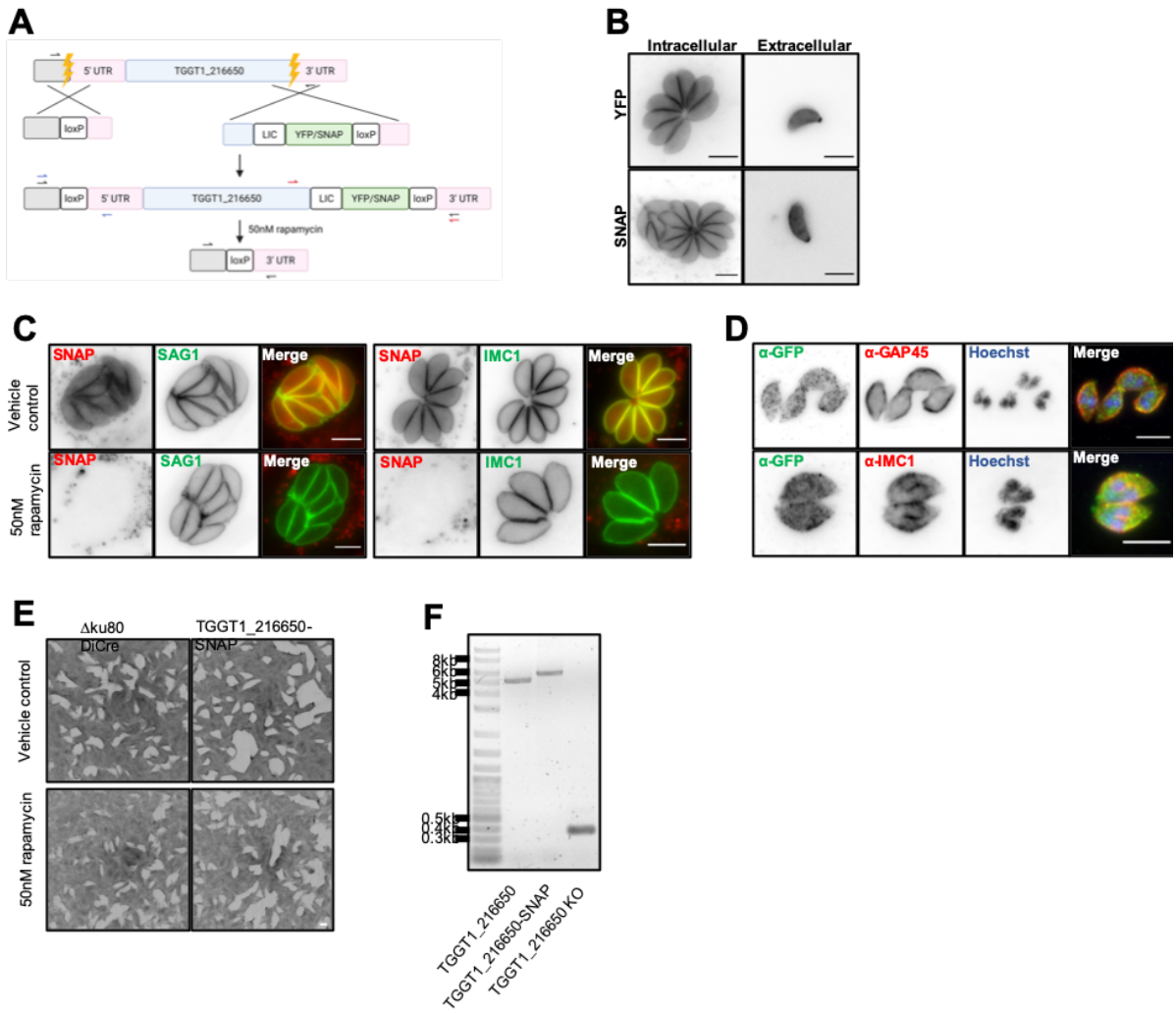
Appendix Figure S2 | **(A)** Clustal Omega Multiple sequence alignment of *Plasmodium spp.* **(B)** Clustal Omega Multiple sequence alignment with the *T. gondii* orthologe. Potential N-terminal palmitoylation site is highlighted in red.

Appendix Figure S3



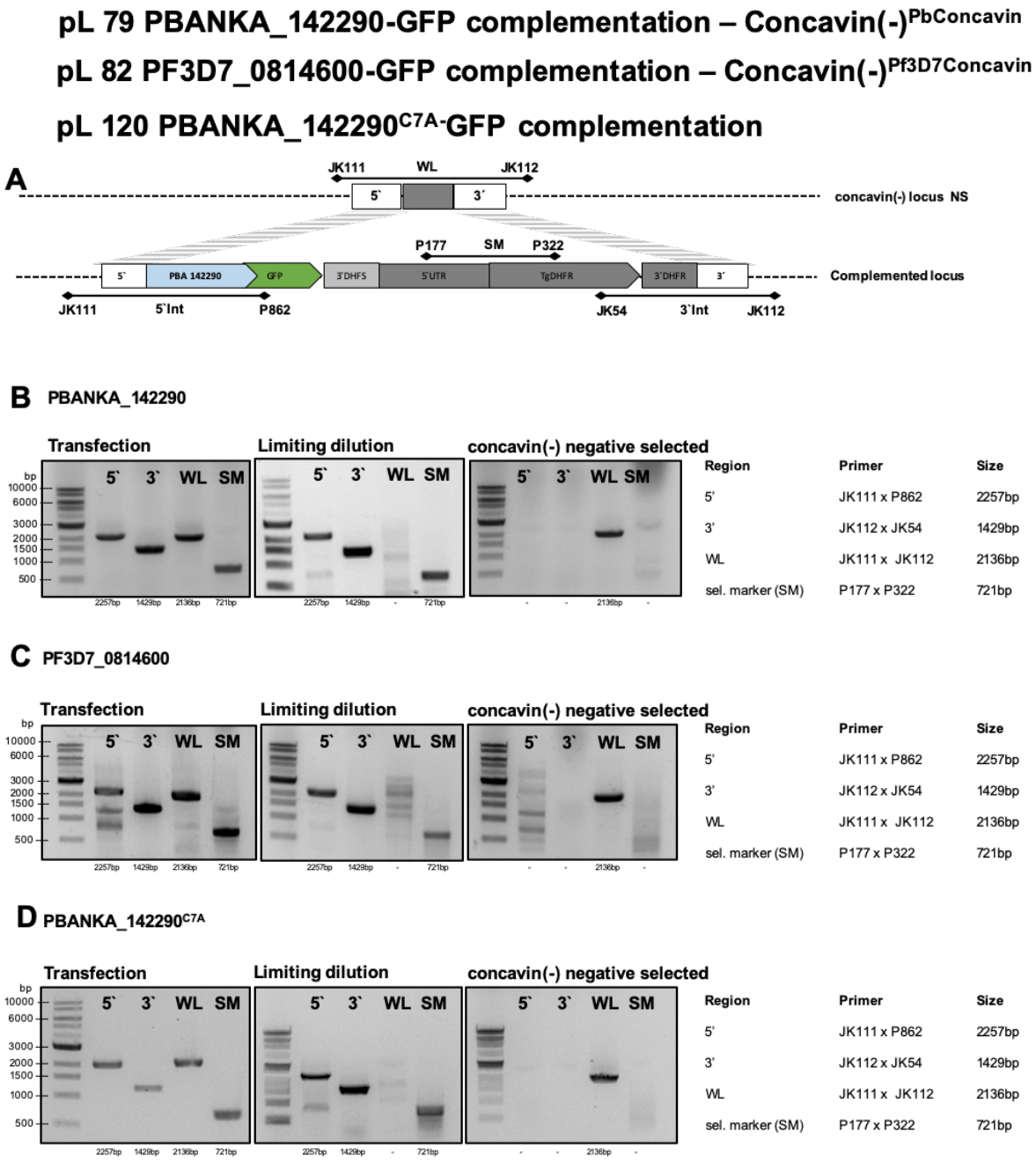
Appendix Figure S3 | Generation of *concavin(-)* and *concavin(-) NS* parasites via double homologous recombination. (A) Cartoon showing the cloning strategy and primers used for genotyping. **(B)** Genotyping PCRs of non-clonal *concavin(-)* parasites directly after transfection and after limiting dilution. Agarose gel pictures show 5`integration, 3`integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. **(C)** Genotyping PCRs of *concavin(-)* parasites after looping out the selection cassette. Expected amplicon sizes are indicated on the right.

Appendix Figure S4



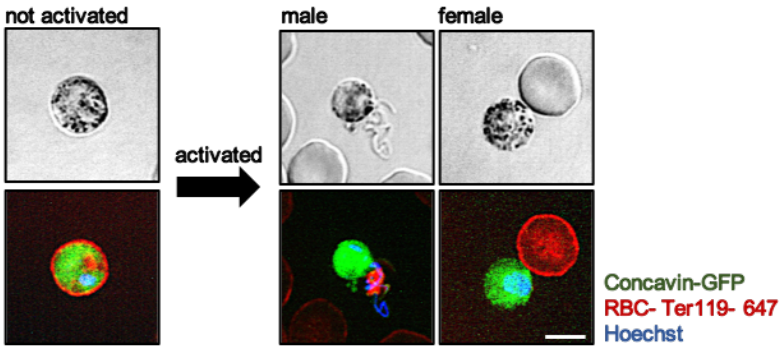
Appendix Figure S4 | TGGT1_216650 is non-essential in *T. gondii* tachyzoites. (A) CRISPR/Cas9 was used to induce double strand breaks. The DNA repair templates used were designed with homology arms to favour homologous recombination. The approximate position of the 5' UTR was estimated based on the TGME49_216650 annotation on ToxoDB. The LIC sequence was used as a linker between the gene and the tags. Correct integration was confirmed via PCR and sequencing, the primer binding sites as indicated with red and blue arrows. Upon addition of 50 nM rapamycin, the Cre recombinase subunits expressed in the parasite strain dimerise, excising the floxed sequence. **(B)** Images show the localisation of TGGT1-216650 endogenously tagged with YFP and SNAP tags. Parasites were imaged live in both intracellular and extracellular conditions. **(C)** SAG1 and IMC1 were internally and C-terminally tagged with HALO-tag respectively using the same protocol as in panel A**.** Upon knockout of TGGT1_216650, no phenotype was observed. The parasites were imaged live. **(D)** The parasites were fixed and antibodies were used to amplify the signal. The gene of interest was not observed at the daughter cells while still inside the mother cell during division. **(E)** 7-day plaque assays. Knockout of the gene of interest following addition of 50 nM rapamycin had no effect on the fitness of the parasites. **(F)** A knockout line was successfully obtained and can be maintained in culture. Confirmation of successful knockout via both PCR and sequencing, the primer binding sites as indicated with black arrows in panel A**.** All scale bars: 5 µm.

Appendix Figure S5



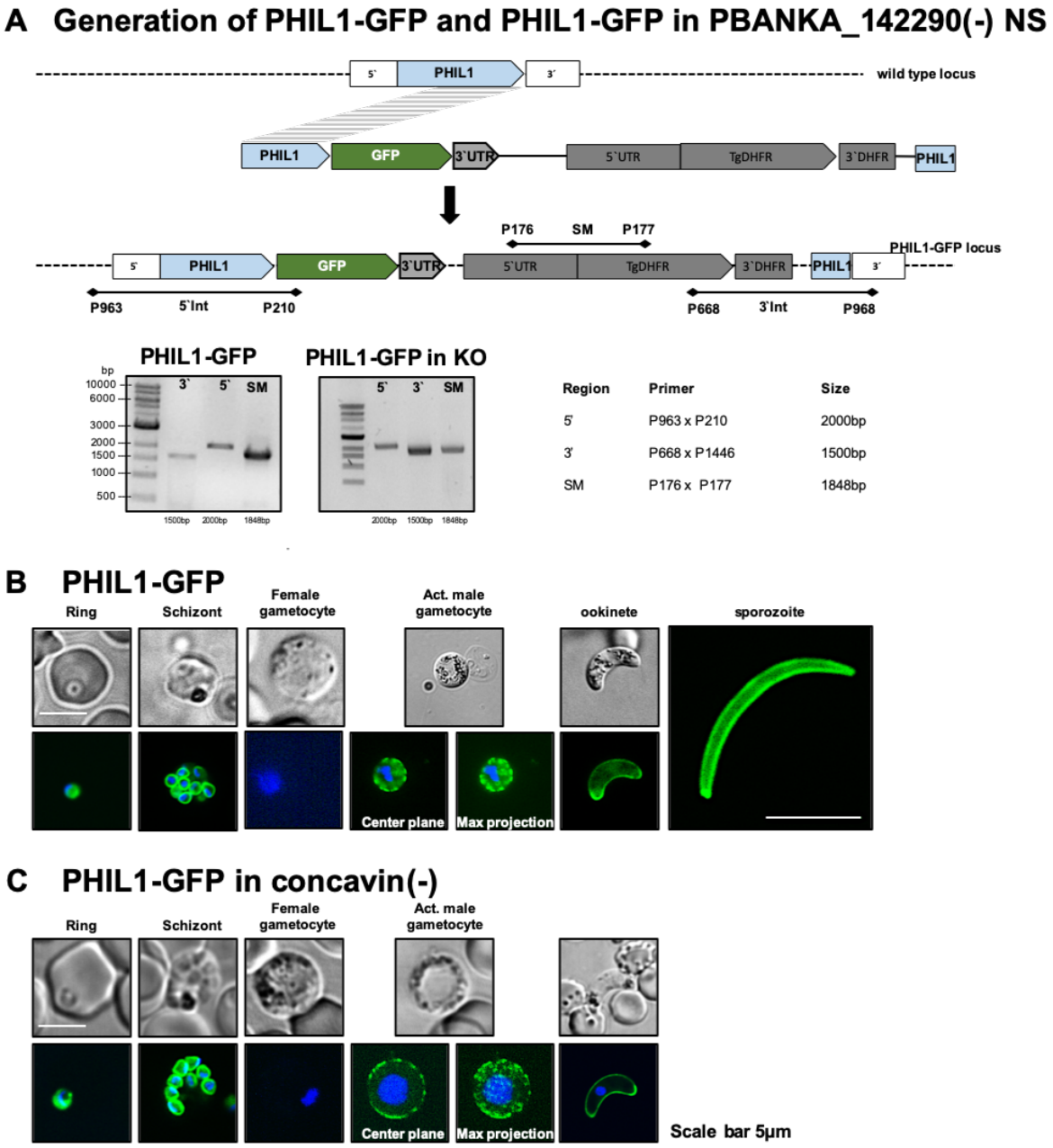
Appendix Figure S5 | Generation of *concavin(-)|P.berghei-gfp*, *concavin(-)|P.falciparum-gfp* and *concavin*^{C7A} parasites via double homologous recombination into *concavin(-)* NS parasites. (A) Cartoon showing the cloning strategy and primers used for genotyping. **(B)** Genotyping PCRs of the non-clonal *concavin(-)|P.berghei-gfp* parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5`integration, 3`integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. **(C)** Genotyping PCRs of the non-clonal *concavin(-)|P.falciparum-gfp* parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5`integration, 3`integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. **(D)** Genotyping PCRs of the non-clonal *concavin*^{C7A} parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5`integration, 3`integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right.

Appendix Figure S6



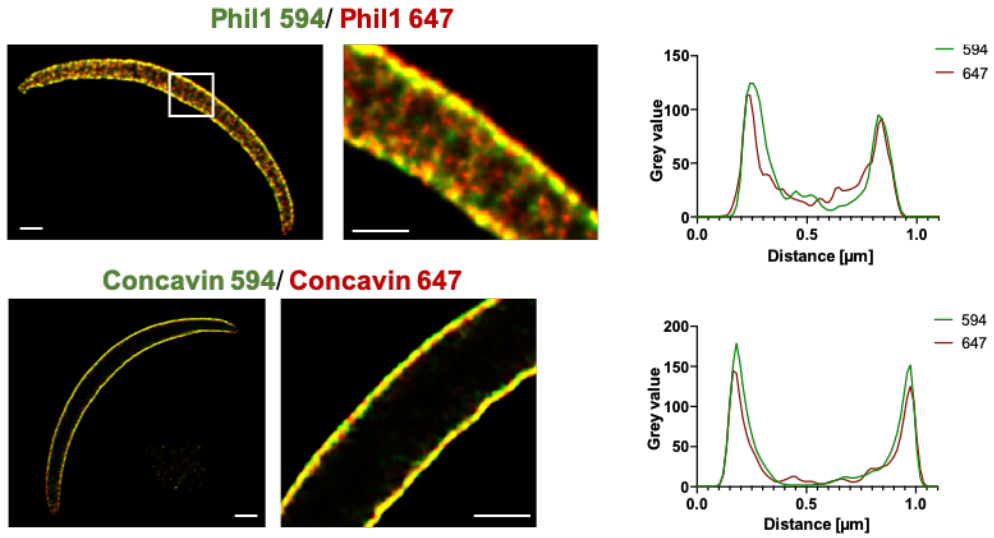
Appendix Figure S6 | Concavin(-)$^{Pbconcavin\text{-}gfp}$ localisation in non- activated and activated gametocytes. Red blood cell membrane is stained with an anti Ter- 119 - 647(red) antibody and nuclei (blue) are stained with Hoechst. Scale bar 5 µm.

Appendix Figure S7



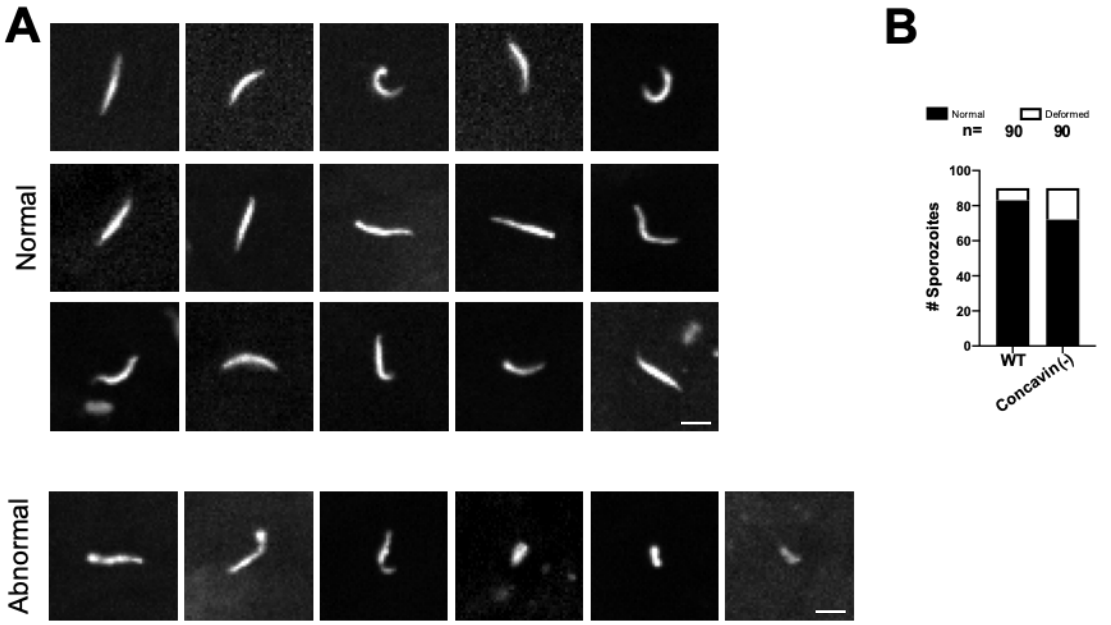
Appendix Figure S7 | **(A)** Generation of *phil1-gfp* and *concavin(-)|phil1-gfp* parasites via single homologous recombination. The cartoon shows the cloning strategy and primers used for genotyping. Agarose gel pictures shows genotyping of the non-clonal parasites in the wild type as well as in the *concavin(-)* background. **(B)** Localization of PhiL1-GFP (green) in wild type parasites. Nuclei (blue) are stained with Hoechst. Scale bar 5 µm. **(C)** Localization of PhiL1-GFP (green) in *concavin(-)* parasites. Nuclei (blue) are stained with Hoechst. Scale bar 5 µm.

Appendix Figure S8



Appendix Figure S8 | Control images used for STED. Bleed through of signal in cells stained with atto-594 into the 647 channel resulted in overlays with almost no difference in distance between the 2 channels. Images were deconvolved using the Richardson-Lucy algorithm. The distance between the 2 signals was measured using the plot profile of the respective channels in Fiji (Figure 3C). Measurements and plot profiles taken at the center of the cell. Scale bar 1 µm.

Appendix Figure S9



Appendix Figure S9 | Examples from a representative bite site after transmission of *concavin(-)* parasites. **(A)** Morphology of normal and abnormal shaped concavin(-) sporozoites at the bite site. Scale bar 5 µm. **(B)** Normally or abnormally shaped WT or *concavin(-)* sporozoites deposited in the skin. 90 sporozoites were observed for both parasite lines.

Appendix Table S1

Primer sequences used for cloning and sequencing.

Name	Sequence
JK 54	AAAGCGGCCGCCGTTTTTCTTACTTATATATTTATACCAA
JK 55	AAAGGTACCCGCATATCCTCATATATAATAAATTACCA
JK 56	TTATCATAAAAGCTTGGCTGTCTT
JK 57	AAAGCGGCCGCAACAAACAAATCTTCATGTTTGT
JK 58	AAACCGCGGTGATATATGTACTCTTTTGTGTTCC
JK 111	AACACCAGTCTGACACCAATTC
JK 112	CCAGATCCAGTATTTTATACCATAGATG
JK 176	GCAGCATTTTCTACTGGATAAGACAG
JK 177	AAATTCGAAATGACAAACATTATAGAATGTACGTTCAAG
JK 178	GGTTCCTTGTCCAATGGATATGACAAAG
JK 179	AAATTCGAATTTGGAATATAACAAAAAAATATATCTCGTAATATA
JK 236	ATGACAAATGTTGTAGAAGCTACTTTTAAAACC
JK 237	CTAGGATCCTTAGGCGCCTTTGTATAGTTCATCCATGCCATGTC
P 136	CGCAATTTGTTGTACATAAAATAGGC
P 176	CTAGACAGCCATCTCCATCTGG
P 177	ATGCATAAACCGGTGTGTCTGG
P 210	TTAACATCACCATCTAATTCAACAAG
P 322	CCCCGTTGTCTGAGAAGG
P 587	CTTTGGTGACAGATACTAC
P 668	TGATTAGCATAGTTAAATAAAAAAAGTTG
P 862	TCCAGTGAAAAGTTCTTCTCCT
P 961	ATCCTCTGGTAATTTTTCG
P 963	TAAGCAGTCGACCTACACAATCATGCATACTATGCC
P 969	TAAGCAGAATTCCCCTTCCGAACAAATTTACGCC
P 970	ATGGATCCaccaccaccaccaccaccaccaccCATATTATCTTTAGGGC
P 1446	CAGCTGCTGGGATTACACATG